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ARTICLE

Plasma Cytokine and Endotoxin Levels Correlate with Survival in Patients with the Sepsis Syndrome

Larry C. Casey; Robert A. Balk; and Roger C. Bone

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Objective: To determine whether plasma tumor necrosis factor-α (TNF-α), interleukin-1 ß (IL-1 ß), interleukin-6 (IL-6), and lipopolysaccharide are detectable in patients when they first present with the sepsis syndrome and to determine whether levels correlate with patient survival.

Design: Prospective study comparing patients with the sepsis syndrome, critically ill patients without sepsis, and normal healthy volunteers.

Setting: Tertiary care hospital affiliated with a medical school.

Patients: The study included 97 consecutive patients on a medical service who met the criteria for the sepsis syndrome; 20 critically ill patients without sepsis who were in the medical intensive care unit; and 20 healthy volunteers who served as comparison groups.

Measurements: Plasma tumor necrosis factor-α, IL-1 ß, interleukin-6, and endotoxin (lipopolysaccharide) levels were measured when a patient was first identified as having the sepsis syndrome. Survival was defined as being alive 30 days after the sepsis syndrome was diagnosed.

Results: Fifty-four percent of patients with the sepsis syndrome had detectable levels of TNF-α (median, 26 pg/mL; range, nondetectable to 1000 pg/mL); 37% had detectable levels of IL-1 (median, 20 pg/mL; range, nondetectable to 2850 pg/mL); 80% had detectable levels of IL-6 (median, 415 pg/mL; range, nondetectable to 2380 pg/mL); and 89% had detectable levels of lipopolysaccharide (median, 2.6; range, nondetectable to 12.5 endotoxin units [EU]/mL). In all cases, levels were higher than those in critically ill patients without sepsis and normal healthy controls ($P < 0.001$ for all comparisons). Plasma levels of TNF-α, IL-1 ß, IL-6, and lipopolysaccharide were detectable in patients regardless of culture status. The IL-6 level was 69% (95% CI, 30% to 108%) higher in patients who died compared with those who survived. The scores for the individual levels of TNF-α, IL-1 ß, IL-6, and lipopolysaccharide were summed to arrive at a total lipopolysaccharide-cytokine score, and mortality increased with lipopolysaccharide-cytokine score ($P < 0.001$).

Conclusions: Patients with the sepsis syndrome have detectable levels of circulating TNF-α, IL-1, IL-6, and lipopolysaccharide independent of culture-documented infection. Lipopolysaccharide and cytokines may play a pathogenic role in sepsis, and the combination of several elevated factors may be important in determining patient survival.

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Sepsis is the 13th leading cause of death in the United States. From 1979 through 1987, sepsis was included in the discharge diagnosis of 2 570 000 hospitalized patients. Furthermore, the incidence of sepsis continues to increase and, despite the use of modern antibiotics and supportive therapy, the mortality rate for patients with the sepsis syndrome remains high (40% to 60%) [1-3].

The cascade of events leading from bacterial infection to the development of organ failure and death are poorly understood. Previous work suggested that a portion of the cell wall of gram-negative bacteria, called lipopolysaccharide, was the agent by which gram-negative bacteria started this cascade, causing organ failure and death [4]. Further evidence supporting a role for lipopolysaccharide in gram-negative sepsis comes from recent multicenter clinical trials that found improved survival in subgroups of patients with sepsis who were treated with monoclonal antibodies to lipopolysaccharide [5, 6].

Recently, it was recognized that lipopolysaccharide is not directly responsible for the adverse sequelae of sepsis. Instead, lipopolysaccharide stimulates the production and release of numerous endogenous mediators, the actions of which are responsible for the pathophysiologic changes and the mortality associated with sepsis. Three cytokines—tumor necrosis factor-α (TNF-α), interleukin-1 ß (IL-1 ß), and interleukin-6 (IL-6)—were recently implicated as important mediators of sepsis.

Tumor necrosis factor-α (also known as cachectin) was the first cytokine implicated in the pathogenesis of sepsis [7]. Intravenous infusion of lipopolysaccharide into laboratory animals or humans causes the appearance of TNF-α in the bloodstream [8, 9]. The C3H/HeJ mouse, which is resistant to lipopolysaccharide, is also genetically deficient in its ability to produce TNF-α. However, this mouse is sensitive to the infusion of exogenous TNF-α [10]. Pretreatment of mice or baboons with antibody to TNF-α improved survival after the infusion of endotoxin or live *Escherichia coli* [11, 12]. Recent clinical studies in patients with meningitis found a positive correlation between high levels of plasma TNF-α and mortality [13]. Multicenter clinical trials using monoclonal antibodies to TNF-α or TNF-α –soluble receptors have recently been completed. These studies implicate TNF-α as an important mediator in sepsis.

Interleukin-1 ß has also been implicated in the pathogenesis of sepsis. Interleukin-1 (IL-1) was previously called endogenous pyrogen, leukocyte endogenous mediator, and leukocyte-activating factor [14]. Infusion of IL-1 causes hypotension, and it acts synergistically with other cytokines to cause tissue injury [15]. Plasma levels of IL-1 ß do not consistently increase in humans who are given intravenous lipopolysaccharide [16] or in patients with sepsis [17]. Recently, the administration of an endogenous IL-1-receptor antagonist in animal models of sepsis improved survival [18], which suggests that IL-1 may be an important mediator of sepsis. Multicenter clinical trials using IL-1-receptor antagonist were recently completed.

Previously called B-cell growth factor, IL-6 is the cytokine that best correlates with mortality in patients with sepsis [19, 20]. The infusion of IL-6 causes no adverse hemodynamic changes [21]. The studies suggest that IL-6 may be a marker of systemic inflammation rather than a mediator of sepsis [19-21].

To determine the relation between plasma levels of TNF-α, IL-1 ß, IL-6, and lipopolysaccharide and outcome in patients with sepsis, we studied lipopolysaccharide and cytokine levels in 97 patients with the sepsis syndrome. The mediators were all elevated in patients with the sepsis syndrome, as compared with critically ill patients without sepsis and normal controls. Elevated levels occurred independent of the presence or absence of culture-documented infection. A score based on the magnitude of the levels of lipopolysaccharide, TNF-α, IL-1 ß, and IL-6 (lipopolysaccharide-cytokine score) was developed and strongly correlated with mortality. These data provide important information about cytokine and lipopolysaccharide levels in patients with the sepsis syndrome when they are first identified and support future clinical trials aimed at determining whether cytokine inhibition may be an alternative strategy for treating patients with sepsis.

Methods

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Patients

The study group included 97 patients with the sepsis syndrome, 20 critically ill patients without sepsis in the medical intensive care unit, and 20 healthy controls. The criteria for inclusion in the group with sepsis syndrome have been previously reported [22] and include fever (temperature >38.3 °C) or hypothermia (temperature <35.5 °C), tachycardia (>90 beats/min), tachypnea (>20 breaths/min), clinical suspicion of infection, and at least one of the following: hypoxemia (PaO_2 <72 mm Hg on room air or $Pa_{O/FI}O_2$ <280), oliguria (urine output <30 mL or 0.5 mL/kg body weight for 1 hour), unexplained metabolic acidosis (or an elevated plasma lactate level), or a recent change in mental status. Patients were identified by a full-time clinical coordinator who surveyed the medical services for patients who met the criteria for the sepsis syndrome. Admission to the medical intensive care unit was not a prerequisite for patient identification. Critically ill patients without sepsis who were in the intensive care unit served as one group of controls; these patients' diagnoses included acute myocardial infarction (n = 4), severe congestive heart failure (n = 7), cardiogenic shock (n = 5), pulmonary embolism (n = 2), and intracranial hemorrhage (n = 2). Laboratory technicians and physicians served as normal, healthy controls (n = 20).

Patients known or suspected to have the acquired immunodeficiency syndrome (AIDS) were excluded from the study. All patients with sepsis were treated by their own physicians using standard therapy for sepsis or septic shock. This study was approved by the Committee for the Protection of Human Subjects from Research Risks at Rush-Presbyterian-St. Luke's Medical Center.

Study Design

Every effort was made to identify patients with the sepsis syndrome as early as possible in their hospital course. This was accomplished through the use of full-time clinical coordinators and through the cooperation of the medical housestaff. Within 30 minutes of the time that a patient was identified as meeting the criteria for the sepsis syndrome, blood (7 mL) was collected into endotoxin-free tubes containing disodium EDTA (ethylenediaminetetraacetic acid). Plasma was separated by centrifugation, aliquoted, and frozen (–70°C) until assayed for lipopolysaccharide and cytokines.

Patient survival was defined as being alive 30 days after meeting criteria for the sepsis syndrome. Hypotension was defined as a systolic blood pressure of less than 90 mm Hg or a decrease of more than 40 mm Hg from the baseline value, where the blood pressure improved with the infusion of intravenous fluids. Shock was defined as hypotension (systolic blood pressure <90 mm Hg or a decrease in systolic blood pressure >40 mm Hg) that was unresponsive to intravenous fluids and occurring in conjunction with an elevated plasma lactate level or unexplained metabolic acidosis with an anion gap.

Plasma Cytokines

Plasma TNF-α concentrations were quantitated using a TNF-α-specific enzyme-linked immunosorbent assay (ELISA) (T Cell Science, Cambridge, Massachusetts) according to the manufacturer's instructions. Samples with high levels of TNF-α were diluted and re-assayed. This assay detected only human TNF-α and the lower limit of detection in our laboratory was 20 pg/mL. This assay detected TNF-α even if it was bound to its soluble receptor. The interassay coefficient of variability was 15%. Recovery of exogenous TNF-α (100 pg/mL) added to EDTA-treated plasma from septic patients with low TNF-α levels was greater than 90%.

Plasma IL-1 ß was measured by ELISA (Citron Biotechnologies, Pine Brook, New Jersey) according to the manufacturer's instructions. This assay detected only IL-1 ß and the lower limit of detection in our laboratory was 20 pg/mL. Recovery of exogenously added 17-kd recombinant human IL-1 (100 pg/mL) from EDTA-treated plasma obtained from patients with sepsis who had low plasma immunoreactive IL-1 levels was greater than 85% (range, 85% to 100%).

Plasma IL-6 was measured using an ELISA (R & D Systems, Minneapolis, Minnesota) according to the manufacturer's instructions. This assay detected only IL-6, and the lower limit of detection in our laboratory was 20 pg/mL. Recovery of recombinant human IL-6 from EDTA-treated plasma from septic patients who had low levels of IL-6 was greater than 90%.

Cytokine Assay Validation

To show the ability of the ELISAs to detect cytokines produced by blood cells in response to lipopolysaccharide, we incubated heparinized whole blood obtained from normal healthy controls with lipopolysaccharide (10 ng/mL). The addition of pyrogen-free saline to heparinized whole blood resulted in no detectable level of TNF-α, IL-1 ß, or IL-6. The addition of lipopolysaccharide (10 ng/mL) caused a time-dependent appearance of cytokines. Plasma levels of TNF-α reached a maximum at 4 hours, with peak levels of approximately 12 000 pg/mL. After 4 hours, the levels decreased, and by 24 hours the levels were frequently nondetectable. Plasma IL-1 ß was nondetectable at time 0 after the addition of lipopolysaccharide, and levels did not begin to increase until 4 hours after the addition of lipopolysaccharide. Maximum levels of IL-1 ß were reached after 24 hours (approximately 6000 pg/mL). Plasma levels of IL-6 were initially nondetectable, had a delayed onset of increase, and reached approximately 20 000 pg/mL after 24 hours.

In this whole-blood system, we also assayed for bioactive TNF (L929 cell lysis). The time course of the appearance of bioactive TNF was identical to that of immunoreactive TNF. The correlation between immunoreactive and bioactive TNF was 0.90.

Lipopolysaccharide Assay

We assessed lipopolysaccharide using a quantitative chromogenic Limulus amebocyte lysate assay (QCL-1000, Whittaker M.A. Bioproducts, Walkersville, Maryland) according to the manufacturer's instructions. Blood was drawn aseptically into lipopolysaccharide-free tubes. Empty blood-collection tubes were selected at random and tested for lipopolysaccharide by placing pyrogen-free water into the tubes and then assaying for lipopolysaccharide. No lipopolysaccharide was detected in the blood-collection tubes containing pyrogen-free water under these conditions. All samples were processed in a laminar flow hood. To minimize nonspecific plasma inhibitors, samples were diluted with pyrogen-free water and heat inactivated at 100 °C for 10 minutes [23]. *Escherichia coli* 055:B5 reference endotoxin (1 endotoxin unit [EU] = 0.6 ng/mL) was used for the standard curve (Whittaker M.A. Bioproducts). The lower limit of detection was 0.1 EU/mL. No differences were observed between standard curves made using diluted heat-inactivated plasma or pyrogen-free water. The presence of bilirubin did not interfere with the assay.

Data Analysis

Statistical calculations were done using Systat. For data that were not normally distributed, the Mann-Whitney U test was used if only two groups were being compared; the Kruskal-Wallis one-way analysis of variance was used if more than two groups were being compared. For normally distributed data, analysis of variance was used. Spearman rank correlation was used to determine if there were correlations between variables. Plasma lipopolysaccharide and cytokine levels were divided into three categories. Stem and leaf plots were used to obtain the median and upper and lower hinges. The median splits the ordered data in half, and the hinges split each half once more. The lower hinge was used to split the data between category 1 and category 2, and the upper hinge to split the data between category 2 and category 3. An lipopolysaccharide-cytokine score was calculated by assigning a value of 0 if the level was in category 1, a value of 2 if the level was in category 2, or a value of 4 if the level was in category 3 (Table 1). The scores for individual levels of TNF-α, IL-1 ß, IL-6, and lipopolysaccharide were then summed to arrive at a total lipopolysaccharide-cytokine score. The associations between mortality and the categorized levels were analyzed using the chi-square test for trends. Levels that were nondetectable were assigned a value equal to the lower limit of detection for the assay. The level of significance was set at $P < 0.05$.

View this table: Table 1. **Lipopolysaccharide-Cytokine Scoring***
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Demographics and Outcome

Of the 97 patients with the sepsis syndrome, 49 were men and 48 were women. The mean age was 60 years (range, 18 to 90 years). Patient survival was 54%. Forty patients had hypotension, and 17 had septic shock; a site of infection was established by culture in 44 patients, of whom 35 had bacteremia. Of the 35 patients with bacteremia, 16 had gram-negative bacteremia, 17 had gram-positive bacteremia, and 2 had both gram-positive and gram-negative bacteremia. Of the 53 patients who did not have culture-documented infection, 38 (71%) had a clinical diagnosis of pneumonia (based on chest radiograph, physical examination, and a Gram stain of the sputum), 10 (19%) had peritonitis, 2 (4%) were thought to have had urosepsis, 1 (2%) had culture-negative endocarditis (echocardiographic demonstration of vegetations on valves), 1 (2%) had cellulitis, and 1 (2%) had pulmonary infarction.

Plasma Cytokines and Lipopolysaccharide

At the time of the identification of patients with the sepsis syndrome, 54% had a detectable level of plasma TNF-α (median, 26 pg/mL; range, nondetectable to 1000 pg/mL), 37% had a detectable level of IL-1 ß (median, 20 pg/mL; range, nondetectable to 2850 pg/mL), 80% had a detectable level of IL-6 (median, 415 pg/mL; range, nondetectable to 2380 pg/mL), and 89% had a detectable level of lipopolysaccharide (median, 2.6 EU/mL; range, nondetectable to 12.5 EU/mL). To determine if plasma levels of TNF-α, IL-1 ß, IL-6, and lipopolysaccharide were elevated in patients with the sepsis syndrome, comparisons were made between critically ill patients without sepsis and normal healthy controls (Figure 1). The mean plasma TNF-α level was higher in patients with the sepsis syndrome (92 pg/mL) than in either the critically ill group (20 pg/mL, $P < 0.001$) or the control group (30 pg/mL, $P < 0.001$). The mean plasma IL-1 ß level was higher in the group with the sepsis syndrome (267 pg/mL) than in the critically ill group (20 pg/mL, $P < 0.001$) or the control group (20 pg/mL, $P < 0.001$). The mean plasma IL-6 level was higher in the group with the sepsis syndrome (730 pg/mL) than in either the critically ill group (519 pg/mL, $P < 0.001$) or the control group (197 pg/mL, $P < 0.001$). The mean plasma lipopolysaccharide level was higher in the group with the sepsis syndrome (3.45 EU/mL) than in the critically ill group (1.80 EU/mL, $P = 0.02$) or the control group (0.04 EU/mL, $P < 0.001$). Thus, patients with the sepsis syndrome have elevated plasma levels of TNF-α, IL-1 ß, IL-6, and lipopolysaccharide as compared with critically ill patients who do not have sepsis or normal, healthy controls.

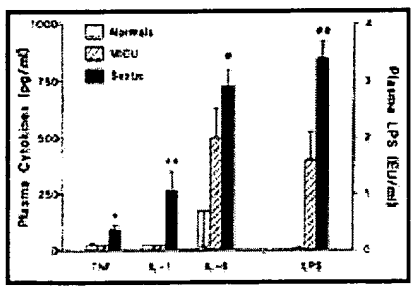

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Figure 1. **Comparison of plasma lipopolysaccharide, tumor necrosis factor-α, interleukin-1 ß, and interleukin-6 levels in patients with the sepsis syndrome ($n = 97$), patients in medical intensive care who did not have sepsis ($n = 20$), and normal healthy controls ($n = 20$).** Plasma samples were obtained immediately after the identification of patients with the sepsis syndrome and critically ill patients without sepsis who were in medical intensive care (MICU). The three groups differed regarding plasma

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levels of tumor necrosis factor-α (TNF), interleukin-1 ß (IL-1 ß), interleukin-6 (IL-6), and lipopolysaccharide (LPS) ($P < 0.001$ for all comparisons). Plasma TNF, IL-1 ß, IL-6, and lipopolysaccharide levels were elevated in patients with sepsis as compared with both critically ill patients without sepsis ($P < 0.01$ for all comparisons) and normal controls ($P < 0.001$ for all comparisons).

Although 53 of the patients had TNF-α that was detected by ELISA, the levels were too low to be detected by bioassay. Only 4 patients had extremely elevated TNF-α as measured by ELISA. Bioactive TNF was present in these samples, but the levels did not correlate with the immunoreactive levels.

Mean plasma levels of lipopolysaccharide, TNF-α, IL-1 ß, and IL-6 for patients with the sepsis syndrome are shown in Table 2 according to culture results. Plasma levels of TNF-α differed among patients according to culture status ($P < 0.01$). Levels of TNF-α were higher in patients with gram-negative bacteremia than in those with gram-positive bacteremia ($P = 0.04$), but patients with gram-positive bacteremia had a higher mortality rate (65% compared with 38%, $P = 0.1$).

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Table 2. **Plasma Tumor Necrosis Factor-α, Interleukin-1 ß, Interleukin-6, and Lipopolysaccharide Levels according to Culture Results***

Mean plasma levels of TNF-α, IL-1 ß, and lipopolysaccharide did not differ ($P > 0.2$) between patients who survived or died (Figure 2). However, the mean plasma IL-6 level was 69% (95% CI, 30% to 108%; $P < 0.01$) higher in patients who died (Figure 2). Because many patients had nondetectable levels of cytokines, the comparison of survivors and nonsurvivors may hide an effect for those patients with elevated levels. To further evaluate the relations among plasma cytokine levels, lipopolysaccharide, and mortality, plasma levels of the mediators were divided into three categories (see Table 1). No relation was observed between the categorized level of lipopolysaccharide and mortality [$P > 0.2$] (Figure 3). Associations were found between categorized levels of TNF-α and mortality ($P = 0.047$), IL-I ß and mortality ($P < 0.01$), and IL-6 and mortality ($P < 0.01$) (Figure 3). Mortality increased with increasing lipopolysaccharide-cytokine score ($P < 0.001$) (Figure 4).

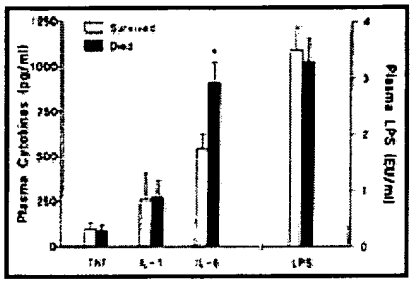

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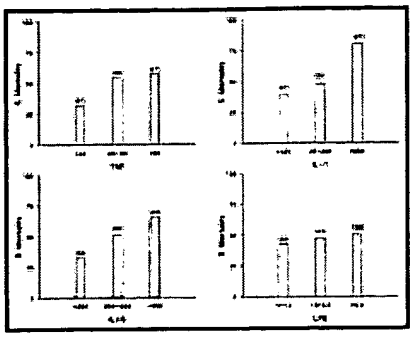
Figure 2. **Plasma tumor necrosis factor-α, interleukin-1 ß, interleukin-6, and lipopolysaccharide levels in patients with the sepsis syndrome who survived or died.** Plasma interleukin-6 (IL-6) was elevated in patients who died ($P < 0.01$). No differences were observed in plasma tumor necrosis factor-α (TNF), interleukin-1 ß (IL-1), or lipopolysaccharide (LPS) levels between patients who survived and those who died ($P > 0.2$).

Figure 3. **Relations between categorized levels of**



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tumor necrosis factor-α (TNF), interleukin-1 ß (IL-1), interleukin-6 (IL-6), and lipopolysaccharide (LPS) (see Table 1 and mortality from the sepsis syndrome. Mortality increased with increasing categorized plasma levels of TNF-α [$P = 0.047$], IL-1 ($P < 0.01$), and IL-6 ($P < 0.01$) but not with lipopolysaccharide level ($P > 0.2$). The number of patients in each category is indicated at the top of each bar.

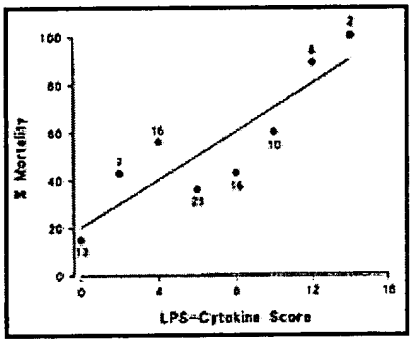

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Figure 4. **Relation between lipopolysaccharide-cytokine score and mortality from the sepsis syndrome.** A unit increase in the lipopolysaccharide-cytokine score was associated with a 5% increase in mortality ($P < 0.001$). The numbers under the points represent the number of patients with that score.

Discussion

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Our study shows that patients with the sepsis syndrome have elevated plasma levels of lipopolysaccharide, TNF-α, IL-1 ß, and IL-6. These elevated levels were independent of the presence or absence of culture-documented infection. The individual cytokine level that best correlated with mortality was the IL-6 level; however, a better correlation was found between lipopolysaccharide-cytokine score and mortality. These data suggest that lipopolysaccharide and cytokines play a role in the pathogenesis of sepsis and that low-level elevations or combinations of lipopolysaccharide and TNF-α, IL-1 ß, or IL-6 may be as important in determining patient survival as are large increases in either the lipopolysaccharide level or the level of any one of the cytokines.

Our data were obtained using sensitive and specific ELISAs for TNF-α, IL-1 ß, and IL-6. Nevertheless, several factors could potentially confound the interpretation of the data: 1) The assays used in our study detect the presence of immunologically reactive material, which may or may not be biologically active; 2) circulating soluble receptors or inhibitors may bind to the cytokine and neutralize its biological activity, which may or may not interfere with the immunologic assay; and 3) other factors in plasma may potentially interfere with the cytokine assays.

Quantitative assays for lipopolysaccharide are notoriously problematic. Because of the sensitivity of the assay, the most common problem is false-positive readings due to sample contamination. Plasma may contain inhibitory

factors that interfere with the assay. Some of these factors (for example, bactericidal permeability increasing protein and some lipoproteins) may bind lipopolysaccharide and modify its activity. The Limulus assay is a complex assay involving several different enzymes. Thus, the quantitation of endotoxin may not be absolute.

In addition to the potential problems with the assays, another important consideration is the time at which the samples were collected relative to the onset of sepsis. In a clinical study, it is not possible to determine the exact time of onset of sepsis. Although we attempted to identify patients when they first developed manifestations of sepsis, it is likely that patients presented at different stages. Thus, we may have missed the transient elevation in plasma TNF-α. Nevertheless, our data represent the cytokine and lipopolysaccharide levels present in patients at the time they are identified as having sepsis, are enrolled in clinical trials, or are begun on therapy.

Despite these limitations in assay method and timely patient identification, we found correlations between mortality and plasma levels of cytokines and lipopolysaccharide. Although this correlation does not imply cause and effect, it does warrant further investigation using selective monoclonal antibodies, soluble receptors, or receptor antagonists as new approaches for treating sepsis. Other cytokines, including IL-8 and IL-10, may also play important roles in sepsis.

The sepsis syndrome consists of a collection of clinical manifestations of sepsis with evidence of organ dysfunction. Patients with this syndrome have a high mortality rate (20% to 30%), independent of the presence of culture-documented infection [2, 3]. In several multicenter clinical trials that included patients with the sepsis syndrome, patients with and patients without culture-documented infection had similar mortality rates [2, 3]. This was also true in our study. We found that patients who did not have culture-documented infection still had elevated plasma lipopolysaccharide, TNF-α, IL-1 ß, and IL-6 levels. Even though blood cultures were negative, it is still likely that these patients were infected. The most common site of infection in our patients with negative cultures was the lung. Sputum cultures are commonly negative and are not a reliable standard for diagnosing pneumonia [24]. Because the infusion of cytokines is known to reproduce clinical manifestations of the sepsis syndrome [25], any disease process—independent of the presence or absence of infection—that induces a systemic inflammatory response by the systemic production of cytokines could produce a clinical picture that resembles the sepsis syndrome. Although infection is the most common cause of a systemic inflammatory response, it is now recognized that burns [26], trauma [27], pancreatitis [28], transplant rejection [29], OKT3 monoclonal antibody administration [30], autoimmune disease [31], and the infusion of cytokines as chemotherapeutic agents can produce a picture that is clinically indistinguishable from the sepsis syndrome.

The plasma levels of TNF-α in our study were consistent with previous findings [9, 13, 32]. Four of our patients did have levels greater than 1000 pg/mL; and, although they were critically ill in the intensive care unit, two of them survived. Similarly, six patients died of septic shock yet had nondetectable levels of TNF. Thus, based on our work and previous reports, TNF-α, by itself, does not appear to be a good marker or predictor of mortality. However, this does not minimize the potential role of TNF-α in patients with sepsis.

In a clinical setting, except in cases of meningitis, the abrupt onset of fulminant gram-negative sepsis in a previously normal patient is uncommon. Although we made every attempt to identify septic patients as early as possible during their clinical course, it is, nevertheless, possible that the time at which blood samples were obtained may not have coincided with the time of peak circulating TNF-α levels. Plasma levels of TNF-α depend on the net balance between its production and disappearance.

Many factors influence the disappearance of circulating TNF: receptor-binding, metabolism, degradation, and neutralization by inhibitors. Mathison and colleagues [8] failed to find a change in the half-life of circulating TNF-α during endotoxic shock. Some studies have shown rapid decreases in circulating levels of TNF-α, primarily in patients with fulminant sepsis secondary to meningitis [13, 19]. Other studies, however, found plasma levels to be very stable over time, even if the levels were persistently elevated [35, 36]. It is not clear if patients with persistently elevated TNF-α levels have increased production, decreased clearance, or both, or if the circulating cytokine is biologically active. Because TNF-α is degraded rapidly, assays that detect biologically inactive fragments may overestimate the level of circulating TNF-α. Finally, the presence of circulating inhibitors, such as

the TNF-α soluble receptor or α_2-macroglobulin, could potentially interfere with TNF assays.

The infusion of IL-1 ß produces hypotension in rabbits [15]. After an intravenous infusion of lipopolysaccharide into humans, the plasma IL-1 ß level increased but only to a maximum of 70 pg/mL [16]. In children with severe infectious purpura [32] and in adults with severe meningococcal meningitis [19], plasma IL-1 ß levels were elevated and correlated with the severity of sepsis. In our study, only 14% of the patients had detectable levels of IL-1 ß. However, we found a correlation between detectable levels and mortality. We also found IL-1 ß levels to be elevated in patients with gram-positive sepsis, although they did not differ from those in patients with gram-negative sepsis. The administration of IL-1-receptor antagonist improved survival in animal models of sepsis [18, 38] and appeared to be promising in the initial clinical trial. The multicenter trial of IL-1-receptor antagonist in septic patients has recently been completed. Our data, in conjunction with previous findings, suggest that IL-1 may be an important marker or mediator of severe sepsis.

Several studies found IL-6 levels to be elevated in patients with sepsis, and the magnitude of the elevation correlated with mortality [19, 20]. Our data, collected in a larger sample of patients with the sepsis syndrome, confirms this observation. "Normal" levels of IL-6 are undetectable with currently available assays. In our normal controls, the mean IL-6 level was elevated because one of them had an IL-6 level of more than 2000 pg/mL. This person was not receiving medications and was in apparently good health. Subsequently, we assayed IL-6 levels in 20 additional normal controls, and IL-6 was nondetectable in all 20.

The most recent study of endotoxemia in patients with septic shock was by Danner and colleagues [39].. They studied 100 patients with septic shock and measured endotoxin levels using a chromogenic Limulus amebocyte lysate assay. The mean peak endotoxin concentration in their study was 4.4 ± 1.2 EU/mL. They found circulating endotoxin in 8 of 14 patients with gram-positive bacteremia.

Using the same assay system, we found that our patients had a mean plasma lipopolysaccharide level of 4.0 ± 0.8 EU/mL. Some patients with gram-positive bacteremia had detectable levels of lipopolysaccharide, and the critically ill patients in the medical intensive care unit had elevated levels of lipopolysaccharide. While these latter patients did not meet the criteria for the sepsis syndrome, they may have had a gram-negative infection. Both the patients with gram-positive sepsis and those in the medical intensive care unit were critically ill; all had either hypotension or shock.

An alternative explanation for the presence of endotoxemia in these patients could be gut translocation of endotoxin or bacteria [40]. Gram-positive products react with the Limulus assay only when they are in very high concentrations [39], and this assay, as used by Natanson and colleagues [41], failed to detect circulating levels of lipopolysaccharide in dogs with severe gram-positive sepsis. Our study, like others, failed to find a relation between the amount of circulating lipopolysaccharide and mortality.

Lipopolysaccharide was the original stimulus used to induce TNF-α production. However, it is now recognized that heat-killed staphylococci, toxic shock syndrome toxin, lipoteichoic acid, viruses, fungi, and parasites are all capable of stimulating TNF-α production. Also, nonmicrobially derived products such as C5a can induce TNF-α synthesis by macrophages [37].

In our study, we found that patients with gram-positive sepsis had detectable levels of TNF-α, IL-1, and IL-6. This is the first clinical study to compare cytokine levels in patients who have gram-positive bacteremia with levels in those who have gram-negative bacteremia. Patients with gram-positive bacteremia had a higher mortality rate than did those with gram-negative bacteremia. Based on our data, we cannot determine whether the increased mortality rate was the result of higher levels of IL-1 or IL-6, or if levels were higher because the patients were more critically ill. Nevertheless, it is clear that gram-positive sepsis, like gram-negative sepsis, is associated with elevated levels of circulating cytokines.

Our study is the first to measure simultaneous levels of lipopolysaccharide, TNF-α, IL-1 ß, and IL-6 in patients with the sepsis syndrome. Simultaneous assessment allowed us to determine whether interactions occurred

between lipopolysaccharide and cytokines. Recent studies support the concept of synergistic interactions between lipopolysaccharide and cytokines. Okusawa and colleagues [15] found that TNF-α and IL-1 ß, when given in doses that individually produced no hemodynamic changes, were able to induce hypotension and severe lung injury when given together. Similarly, when lipopolysaccharide was combined with TNF-α in doses that were tolerated individually, they caused lethal shock in mice [42]. In the same study, these two agents were shown to cause hemorrhagic necrosis of the skin when given together subcutaneously; necrosis was not seen when they were injected separately. Other examples of synergy among lipopolysaccharide, TNF-α, and IL-1 ß include protection from fatal hyperoxia [43] and the stimulation of fibroblast prostaglandin E_2 production [44]. In our study, the variable that best correlated with mortality was the lipopolysaccharide-cytokine score. These data suggest that combinations of lipopolysaccharide and cytokines, even at low levels, are as much of a risk factor as are large increases in the levels of any one factor. Our data also show the importance of "profiling" cytokines rather than monitoring the level of only a single type. However, our data suggest that the IL-6 level should be chosen for such single-level monitoring because it had the best correlation with mortality. The ability of the lipopolysaccharide-cytokine score or of the IL-6 level to predict mortality still requires confirmation.

In summary, we found that plasma lipopolysaccharide, TNF-α, IL-1 ß, and IL-6 are elevated in patients with the sepsis syndrome as compared with critically ill patients in the intensive care unit or normal controls. The elevated levels of these mediators in patients with the sepsis syndrome occurred independent of the presence or absence of culture-documented infection. Both gram-positive and gram-negative infections cause elevated circulating levels of TNF-α, IL-1 ß, and IL-6. Although the IL-6 level correlated with mortality, the lipopolysaccharide-cytokine score had the best correlation with mortality. These data provide important information about lipopolysaccharide and cytokine levels in patients with the sepsis syndrome when they are first identified and support future clinical trials aimed at determining whether cytokine inhibition may be an alternative strategy for treating patients with sepsis.

Abbreviations

IL-1ß: interleukin-1ß

IL-6: interleukin-6

TNF-α: tumor necrosis factor-α

Author and Article Information

From Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois.
Requests for Reprints: Larry C. Casey, MD, PhD, Rush-Presbyterian-St. Luke's Medical Center, Department of Pulmonary and Critical Care Medicine, 1725 West Harrison Street, Suite 306, Chicago, IL 60612.

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